Great Britain 2145112A (GB '112) discloses a method of sorting living spermatozoa using a fluorochrome dye and a fluorescence activated cell sorter. GB '112 discloses an ability to sort sperm speculatively on the basis of sperm type with an efficiency of 3.5:1 of correct sex to incorrect sex, or with 72% efficiency. GB '112 does not teach or suggest sorting viable sperm populations for surgical insemination with an efficiency of about 90% in sorting X from Y sperm (specification, pages 8-11) as disclosed in the present application.

Applicant achieves this high degree of sperm sorting efficiency through the employment of an incubation step in conjunction with sperm staining. The present claims require that dye staining be conducted in the temperature range of about 30°-39° C. See, *e.g.*, Claims 9, 14-16, and 27. This temperature range is critical for the purpose of obtaining effective staining to achieve discrimination between Y-bearing sperm and X-bearing sperm while preserving sperm viability. At these temperatures, the DNA becomes uniformly stained (page 7, line 3 of the specification), thereby enhancing the sensitivity of the selection and sorting based upon the differential amount of DNA in X and Y sperm.

GB '112 discloses staining sperm for 2 hours at 20°-22° C (page 2, line 38) and achieves sperm sorting of 72% efficiency. GB '112 does not disclose or suggest sperm staining at 30°-39° C

to obtain effective sperm staining to achieve better discrimination between sperm types or to achieve more efficient sperm sorting.

Claim 9 also addresses a preferred embodiment of the invention which includes a step wherein two detectors are arranged such that the first detector determines orientation of the sperm on the basis of magnitude of fluorescence and controls a second detector to measure the DNA content of those sperm that have been determined to be in a preselected orientation. This dual detector system with its inherent advantages is neither disclosed nor suggested by GB '112.

The claims also address several additional embodiments of the invention not disclosed or suggested by GB '112. For example, applicant may use hydrodynamic forces to properly orient the sperm in the flow of sheath fluid before they are passed before the light source (Claims 19 and 26, specification page 5, lines 13-16), thereby further enhancing efficiency. Hydrodynamic sperm orientation is not disclosed by GB '112.

Additionally, applicant may employ an ultrasonic transducer to regulate the flow of sperm as uniform droplets containing single sperm into the cell sorting means (Claim 24, specification page 5, lines 18-19). The use of an ultrasonic transducer to regulate the flow of sperm into the cell sorting means and the advantages thereof are neither disclosed nor suggested by GB '112.

Applicant also may employ an electronic gating system to remove sperm which are not properly oriented before the sperm sample is passed through the light source (Claims 23 and 26, specification page 5, lines 10-12). GB '112 neither discloses nor suggests the use of an electronic gating system to remove sperm which are not properly oriented in its method of sperm sorting method.

Furthermore, GB '112 is drawn to a method for staining spermatozoa with a fluorochrome dye (*e.g.*, Hoechst 33342) and then sorting the spermatozoa in accordance with the fluorescence profile (page 2, lines 37 *et seq.*). In the British reference, nonmotile bull spermatozoa are shown to display a higher fluorescence than motile spermatozoa, and the profile of motile spermatozoa is shown to be bimodal. The motile spermatozoa were separated into two populations (AI and AII). The fluorescence of the AII population was shown to be approximately 15% greater than that of the AI population (page 3, lines 45-48). Additionally, the patent concludes that the observed bimodality is indicative of two physiologically or biologically different subpopulations of viable spermatozoa; but it is only able to speculate on the significance of the difference (page 4, lines 38-44). The reference states:

> The subpopulations (AI and AII) may reflect spermatozoa at distinct stages of late maturation or the difference between X- and Y-chromosome-bearing spermatozoa (*sic*).

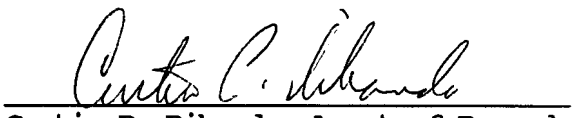
Subsequent statements that experimental work with rabbits resulted in a 3.5:1 ratio of correct sex to incorrect sex and that the disclosed method in the patent has application in sorting spermatozoa according to whether they are X- or Y-chromosome-bearing, cannot be given much credence in view of the speculative statement set forth above. Moreover, it is well established in the art that the difference between fluorescence of the two populations of sperm as established by flow cytometrical, biochemical, and chromosome length data should be on the order of about 3.0-4.2% (3.0% for rabbit, 3.6% for boar, 3.8% for bull, and 4.2% for ram sperm) [Johnson and Clarke, *Gam. Res.* 21: 335 (1988)], rather than the 15% observed by GB '112. This data tends to further cast doubt on the correlation between the observed bimodality in the patent and the actual sorting based on sex.

The Examiner has relied upon Lang to teach that it is conventional to use sorted sperm to alter sex ratios in desired mammals. Notwithstanding, Lang fails to suggest the specific method of dye staining and cell sorting recited in the claims and discussed above. Rather, Lang uses a charge-bearing material in conjunction with the spermatozoa and attempts to sort the X- and Y-bearing spermatozoa in an electrostatic field. In studies with bull and rabbit spermatozoa, this technique resulted in a sex ratio of offspring no greater than about 1.6:1, or an efficiency of 62%.

In contrast, the applicant was able to obtain a distibution as high as 15:1 (Table II, "Sorted X"). It would not be obvious from the teachings of Lang that either the method of GB '112, or a modification of the method of GB '112, would yield an efficiency in sperm sorting with a sex ratio comparable to that obtained by the applicant.

In view of the applicant's foregoing amendments and remarks, it is respectfully submitted that Claims 9-27 be allowed.

Respectfully submitted,



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